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Short Communication

Role of *PTCH* and *p53* Genes in Early-Onset Basal Cell Carcinoma

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Basal cell carcinoma (BCC) is the most common skin cancer in the Western world. Ultraviolet (UV) exposure, race, age, gender, and decreased DNA repair capacity are known risk factors for the development of BCC. Of these, UVB irradiation from sunlight is the most significant risk factor. The incidence of sporadic BCC increases in individuals older than age 55, with the greatest incidence reported in individuals who are older than 70, and is rare in individuals who are younger than 30. In this study, we analyzed 24 BCC samples from individuals who had BCC diagnosed by the age of 30. Fifteen single-stranded conformation polymorphism variants in the *PTCH* gene were identified in 13 BCC samples. Sequence analysis of these single-stranded conformation polymorphism variants revealed 13 single nucleotide changes, one AT insertion, and one 15-bp deletion. Most of these nucleotide changes (nine of 15) were predicted to result in truncated *PTCH* proteins. Fifteen *p53* mutations were also found in 11 of the 24 BCC samples. Thirty-three percent (five of 15) and 60% (nine of 15) of the nucleotide changes in the *PTCH* and *p53* genes, respectively, were UV-specific C→T and CC→TT nucleotide changes. Our data demonstrate that the *p53* and *PTCH* genes are both implicated in the development of early-onset BCC. The identification of UV-specific nucleotide changes in both tumor suppressor genes suggests that UV exposure is an important risk factor in early onset of BCC. *(Am J Pathol 2001, 158:381–385)*

Basal cell carcinoma (BCC) is the most common skin cancer in the Western world.[1,2] It is classified, together with squamous cell carcinoma, as nonmelanoma skin cancer. BCC represents 75% of all nonmelanoma skin cancer. Known risk factors that contribute to the development of BCC include ultraviolet (UV) exposure, race, age, gender, and DNA repair capacity.[3] UVB irradiation, from sunlight, is thought to be the major factor responsible for the development of BCCs, producing DNA damage at those sites where the pyrimidine of the base pair is part of a dipyrimidine sequence. C→T transitional changes at pyrimidine sites, including CC→TT double-base changes, are the most frequent form of nucleotide base substitution at the UVB-damaged dipyrimidine sites.

p53 mutations have been shown in 30 to 50% of BCCs studied, and more than half of these mutations were UV-specific C→T or CC→TT changes.[4–9] These UV-specific changes in the *p53* gene have also been detected in DNA from normal, sun-exposed skin.[10,11] The human homologue of the *Drosophila patched* gene, *PTCH*, was first isolated by two independent groups during their search for the gene responsible for Nevoid basal cell carcinoma syndrome.[12,13] Sequence analysis of DNA from Nevoid basal cell carcinoma syndrome individuals showed a series of germline mutations in the *PTCH* gene. Subsequently, somatic mutations in the *PTCH* gene were identified in 20 to 30% of the sporadic BCCs studied.[12–18] Mutations detected in the *PTCH* genes from sporadic BCCs also contained UV-specific C→T and CC→TT nucleotide changes. Most of the *PTCH* mutations detected have been nonsense mutations, deletions, and insertions that lead to a premature termination of *PTCH* proteins.[17]

An individual's DNA repair ability is thought to play a role in the development of BCC. A rare inherited disorder,

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xeroderma pigmentosum (XP), provides a human model that underscores the important role of DNA repair in preventing human cancers. Individuals with XP are unable to repair DNA damage[19] and are 2,000 times more likely than normal individuals to develop sunlight-related BCC at an early age.[20] Recently, a group studying 22 BCCs from patients with XP identified high levels (>60%) of UV-specific mutations in the *PTCH* gene.[21] Epidemiological studies have also shown that individuals with BCC have a decreased ability to repair UV-induced DNA damage compared to control individuals without BCC.[22]

The incidence of sporadic BCCs increases in individuals older than age 55, with the greatest incidence reported in individuals who are older than 70 years old. BCC is rare in individuals who are younger than 30 years old. In a study conducted in a defined population in a city in southern Sweden, only 12 of 249 (4.8%) BCC cases were from individuals younger than 30 years old.[23] Although some of these patients may have had a history of significant UV exposure, the development of BCCs at this early age is unusual. We postulated that mutations in the *PTCH* and *p53* genes might have contributed to the development of skin cancers in this young population. To test that notion, we retrieved 24 paraffin blocks from 24 early onset BCC cases. Genomic DNA from these BCC samples was subjected to mutation analysis of the tumor suppressor genes *PTCH* and *p53*.

Materials and Methods

Study Participants

Biopsy specimens with a confirmed pathological diagnosis of BCC from individuals who were younger than 30 years old at the time of biopsy were obtained from the Dermatopathology Laboratory at Columbia University. Each individual's age, ethnic background, and medical history were reviewed by their dermatologist and none of these individuals has Nevoid basal cell carcinoma syndrome. The study was approved by the Columbia-Presbyterian Medical Center Institutional Review Board.

DNA Extraction

Paraffin sections containing >50% tumor tissue were placed into a 1.5-ml microcentrifuge tube and washed with xylene (three times for 30 minutes each). The sections were then digested in a buffer with proteinase K (provided in the QiAamp tissue kit; Qiagen, Valencia, CA) at 55°C overnight. The genomic DNA was then extracted following the instructions and columns provided by the QiAamp Tissue Kit from Qiagen. Genomic DNA of the peripheral blood from 20 normal individuals was used as normal control.

Single-Stranded Conformation Polymorphism (SSCP) Analysis

SSCP-polymerase chain reaction (PCR) reaction mixtures containing 25 ng of each primer, 22.5 μl of platinum PCR supermix (Life Technologies, Inc., Rockville, MD) and 0.5 μl of [^{33}P]dCTP (Dupont-NEN, Boston, MA) were subjected to 30 cycles of PCR amplification. After thermal cycling, 1 μl of PCR product was added to 10 μl of stop solution (95% formamide, 10 mmol/L NaOH, 0.25% bromophenol blue, and 0.25% xylene cyanol). The mixtures were heated to 94°C for 3 minutes and placed on ice immediately. Three microliters of the denatured mixtures were loaded onto a 0.5× mutation detection enhancement gel (FMC Bioproducts, Rockland, ME) with 10% glycerol and run at 10 W for 20 hours at room temperature. PCR products with SSCP variants were sequenced using a BigDye terminator cycle sequencing kit (ABI) and were then run on an Applied Biosystems (Foster City, CA) 310 automated sequencing system.

Table 1. Primers for the *p53* Gene

Exon 4	AATGGATGATTTGATGCTGTCCC
	CTCAGGGCAACTGACCGTGC
Exon 5	TTCCTCTTCCTGCAGTACTC
	GCCCCAGCTGCTCACCATCG
Exon 6	CTGATTGCTCTTAGGTCTGG
	AGTTGCAAACCAGACCTCAG
Exon 7	GTGTTGTCTCCTAGGTTGGC
	AAGTGGCTCCTGACCTGGAG
Exon 8	AGTGGTAATCTACTGGGACG
	ATTCTCCATCCAGTGGTTTC

Mutation Screening for the PTCH Gene

A set of 20 pairs of primers flanking exon 3 to exon 23 of the *PTCH* gene, as previously described,[13,24] was used to amplify tumor genomic DNA. These PCR amplicons were then subjected to a SSCP-PCR reaction with the nested primers. All mutations were confirmed by new PCR and sequence reactions starting from DNA.

Mutation Screening for the p53 Gene

Every tumor sample was screened for mutations in the exon 4 to exon 8 of the *p53* gene by direct sequencing analysis. Genomic DNA from each case was subjected to PCR amplification with primers flanking each exon of the *p53* gene. The resulting amplicons were then sequenced and analyzed on an Applied Biosystems model 310 DNA sequencer. Table 1 lists the *p53* primers used. Mutations were confirmed by new PCR and sequence reactions.

Results

In this study, we examined 24 cases of BCC from individuals with a confirmed diagnosis of BCC before the age of 30. All individuals were Caucasians and ranged in age from 16 to 29 years (Table 2). Fifty-four percent (13 of 24) of the BCC samples were from the face and neck (the most common sites for sporadic BCC), and the remaining samples were from the trunk.

Genomic DNA isolated from each BCC sample was first subjected to a PCR-SSCP screening for mutations in the *PTCH* gene. A total of 15 SSCP variants were de-

Table 2a. *PTCH* and *p53* Mutations Identified in BCC Samples

				PTCH			*p53*		
Sample no.	Gender	Age	Site	Exon	Nucleotide*	Effect	Exon	Nucleotide*	Effect
BCC1	F	28	Forehead	8	1093 cC→cT	Q365X	8	833 cC→cT	P278L
BCC2	M	26	Chest	9	1249 tC→tT	Q417X	8	833 cC→cT	P278L
							8	844 cC→cT	R282W
BCC3	F	29	Forehead	15	2308 cC→cT	R772X	6	585 CC→TT	R190X
BCC4	F	28	Chest	14	2062 gC→gT	Q688X			
				9	1292 ccT→ccA	L431Q			
BCC5	F	25	Chest	5	707 Gg→Ag	W236X	7	722 Cc→Tc	S241F
				21	3487 cG→cA	G1163S	8	836 gG→gA	G279E
BCC6	F	29	Nasal fold	18	3054 gG→gA	W1018X	6	640del8	
BCC7	M	27	Neck	13	1729-1 Gg→Tg	Splice			
BCC8	F	25	Left back	17	2709insAT	frameshift			
BCC9	F	26	Scalp	15	2385del15	del5AA	8	839 aGa→aAa	R280K
BCC10	F	28	Arm	13	1847 Gc→Ac	S616N	8	888CC→TT	H297Y
							8	890delC	
BCC11	F	25	Upper lip	5	709 gG→gA	E237K	4	292 cC→cT	P98S
							7	743 cG→cA	R248Q
BCC12	F	18	Upper lip	19	3196 cG→cT	E1066M			
BCC13	F	28	Chest	14	2004 gC→gT	Y668Y	7	722 Cc→Tc	S241F
BCC14	F	21	Preauricular				6	620del8	
BCC15	F	19	Chest				8	844 cC→cT	R282W

*Nucleotide in small case indicates the adjacent sequence.

Table 2b. BCC Samples with Wild-Type *PTCH* and *p53* Genes

BCC16	M	27	Neck
BCC17	F	16	Chest
BCC18	F	28	Cheek
BCC19	F	26	Right upper eyelid
BCC20	M	22	Neck
BCC21	M	27	Behind the ear
BCC22	F	22	Neck
BCC23	F	29	Back
BCC24	F	29	Right upper arm

tected in 13 BCC samples. Sequence analysis of PCR products containing the SSCP variants revealed 15 sequence alternations spanning the entire *PTCH* gene. Twelve of the 15 sequence alternations were single nucleotide changes, resulting in six nonsense mutations, five missense mutations, and one silent mutation. In addition, two frameshift mutations and one in-frame mutation were detected. These consisted of a splice site mutation in exon 13, an AT insertion in exon 17, and a 15-bp deletion in exon 15, respectively. These three mutations, together with the six nonsense mutations, give rise to truncated *PTCH* proteins (Table 2).

Five missense mutations were detected spanning exons 4 to 21 of the coding sequences of the *PTCH* gene (Table 2 and Figure 1). Four of these five missense mutations were in or near transmembrane domains. Eleven of the 12 single nucleotide changes detected occurred at the dipyrimidine sites. Of 12 single nucleotide changes, five C→T and five G→A transitional changes were detected. They were consistent with UV-induced sunlight damage. We also detected a C→T transitional change at nucleotide 2004 in exon 14, resulting in a silent mutation (tyrosine to tyrosine substitution at codon 668). All five missense mutations and the one silent mutation were not detected in 40 normal control alleles, suggesting that they were not sequence polymorphisms.

Sequence analysis from exon 4 to exon 8 of the *p53* gene, was then performed on DNA from the same 24 BCC samples. Fifteen sequence alterations were identified in the coding sequences of the *p53* gene from 11 BCC samples. Three BCC samples had two *p53* mutations each. Twelve of the 15 sequence alterations were single and tandem nucleotide changes that resulted in 11 missense mutations and one nonsense mutation. Nine of the 12 single nucleotide changes were UV-specific C→T and CC→TT changes (Table 2 and Figure 1). Two 8-bp deletions in exon 6 and a 1-bp deletion in exon 8 of the *p53* gene were detected in three BCC samples (Table 2). Nine of the 11 BCC samples with *p53* mutations also contained *PTCH* mutations and all nine of these BCC samples contained UV-specific nucleotide changes in one or both tumor suppressor genes (Table 2).

Discussion

Sporadic BCC has the highest incidence in white males between the ages of 65 and 80. In this study, we evaluated a group of individuals who had their first BCC diagnosed before the age of 30. Because the incidence of sporadic BCC in this age group is very low, few studies have focused on this patient population. We screened 24 BCC samples from 24 individuals for mutations in the *PTCH* and *p53* genes. All 24 individuals were Caucasians between the ages of 16 to 29. The site distribution of the BCC was 54% on the face and neck, and 46% on the trunk. This group of younger individuals has a lower frequency of BCC of the face and neck than the older

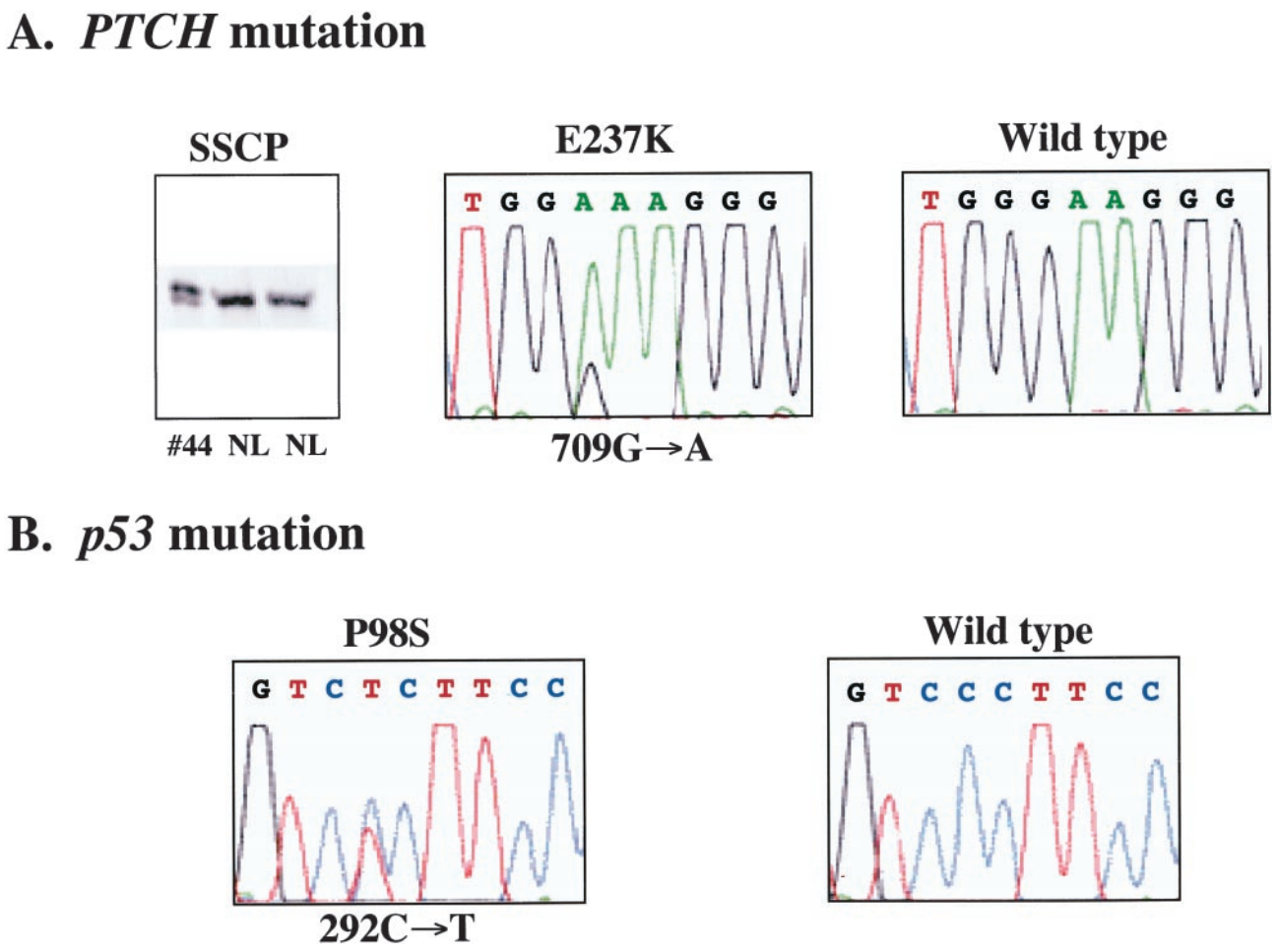

Figure 1. Identification of missense mutations in the *PTCH* and *p53* genes from BCC11 (no. 44). SSCP analysis showed a shifted band in PCR product encoding exon 5 of the *PTCH* gene from sample no. 44 (BCC11). Sequence analysis of this PCR product revealed a G→A change at nucleotide 709 that resulted in a glutamic acid to lysine substitution at codon 237. Direct sequence analysis of the *p53* gene in the same BCC sample revealed an UV-specific, C→T transitional change at nucleotide 292 that resulted in a proline to serine substitution at codon 98. NL, wild-type sequence from normal tissue.

individuals; >80% of the BCCs reported in the 65 to 80 age group are located on the face and neck.[25] Moreover, >79% (19 of 24) of individuals in our early-onset group were female, in contrast to the older age group where the gender distribution is approximately equal.[26]

Fifty-four percent (13 of 24) of the BCC samples in this study had mutations in the *PTCH* gene, compared to an average *PTCH* mutation rate of 35% in BCC samples from older populations.[13–15,17,18] The difference is statistically significant by chi-square test ($P < 0.05$). Of the 15 nucleotide changes in the *PTCH* gene, 80% (12 of 15) were point mutations. Our results differ from those reported in published studies on sporadic BCC in older age groups,[14] where 47% (nine of 19) of the nucleotide changes were point mutations. Our data are similar to that of a recent study in BCC from individuals with XP. In that study, 73% (16 of 22) of BCC samples contained *PTCH* mutations, and 89% of them were point mutations.[21] It has been suggested that decreased DNA repair ability in XP individuals contributes to the high frequency of *PTCH* mutations and high level of point mutations.[21] Based on our findings, we speculate that early onset BCC results from a reduced ability to repair DNA damage. Using individuals without BCC as normal controls, Wei and colleagues[22] showed that a combination of reduced DNA repair ability and exposure to UV irradiation was associated with an increased risk of BCC.

The *PTCH* gene encodes two large extracellular loops and 12 transmembrane domains that binds to *sonic hehedgehog*, a member of the *hedgehog* gene family.[27] The two large extracellular domains of the *PTCH* gene are required for this binding.[29] In this study, four of the six sequence alterations that led to five missense mutations and one silent mutation, were located in the transmembrane domains. The remaining nine nucleotide changes that we observed are predicted to result in a truncated *PTCH* protein. The mutations seen in our population differ from those seen in Nevoid basal cell carcinoma syndrome individuals, in whom most of the *PTCH* germline mutations reported were insertions and deletions.[28]

In our group of BCC patients, we found 15 *p53* mutations in 11 BCC samples. Sixty percent (nine of 15) of these mutations were UV-specific C→T and CC→TT changes. The increased incidence of point mutations in *PTCH* coupled with UV-specific mutations in *p53* suggests that the young individuals in our patient population

may have a decreased ability to repair UV-induced DNA damage. Overall, we showed that 37% (nine of 24) of our BCC samples had mutations in both *PTCH* and *p53* genes. Three BCC samples had only *PTCH* mutations, and two BCC samples had only *p53* mutations.

In conclusion, we have found a series of mutations in the *PTCH* and *p53* genes in BCC samples from a group of individuals with early onset BCC. We have shown that similar to previous studies in older age population, UV irradiation plays a major role in the development of BCC in a young population. We speculate that these young individuals have decreased DNA repair ability that renders them more susceptible to UV-induced DNA damage and, therefore, prone to develop BCC at a younger age. Further studies are needed to assess DNA repair ability in this population and to evaluate the role of other risk factors in the development of BCC in young individuals.

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References

1. Miller SJ: Biology of basal cell carcinoma (part I). J Am Acad Dermatol 1991, 24:1–13
2. Gloster Jr HM, Brodland DG: The epidemiology of skin cancer. Dermatol Surg 1996, 22:217–226
3. Preston DS, Stern RS: Nonmelanoma cancers of the skin. N Engl J Med 1992, 327:1649–1662
4. Rady P, Scinicariello F, Wagner Jr RF, Tyring SK: p53 mutations in basal cell carcinomas. Cancer Res 1992, 52:3804–3806
5. Moles JP, Moyret C, Guillot B, Jeanteur P, Guilhou JJ, Theillet C, Basset-Seguin N: p53 gene mutations in human epithelial skin cancers. Oncogene 1993, 8:583–588
6. Ziegler A, Leffell DJ, Kunala S, Sharma HW, Gailani M, Simon JA, Halperin AJ, Baden HP, Shapiro PE, Bale AE, Brash DE: Mutation hotspots due to sunlight in the p53 gene of nonmelanoma skin cancers. Proc Natl Acad Sci USA 1993, 90:4216–4220
7. van der Riet P, Karp D, Farmer E, Wei Q, Grossman L, Tokino K, Ruppert JM, Sidransky D: Progression of basal cell carcinoma through loss of chromosome 9q and inactivation of a single p53 allele. Cancer Res 1994, 54:25–27
8. Kubo Y, Urano Y, Yoshimoto K, Iwahana H, Fukuhara K, Arase S, Itakura M: p53 gene mutations in human skin cancers and precancerous lesions: comparison with immunohistochemical analysis. J Invest Dermatol 1994, 102:440–444
9. D'Errico M, Calcagnile AS, Corona R, Fucci M, Annessi G, Baliva G, Tosti ME, Pasquini P, Dogliotti E: p53 mutations and chromosome instability in basal cell carcinomas developed at an early or late age. Cancer Res 1997, 57:747–752
10. Kanjilal S, Strom SS, Clayman GL, Weber RS, el-Naggar AK, Kapur V, Cummings KK, Hill LA, Spitz MR, Kripke ML, Anauthaswamy HN: p53 mutations in nonmelanoma skin cancer of the head and neck: molecular evidence for field cancerization. Cancer Res 1995, 55:3604–3609
11. Ponten F, Berg C, Ahmadian A, Ren ZP, Nister M, Lundeberg J, Uhlen M, Ponten J: Molecular pathology in basal cell cancer with p53 as a genetic marker. Oncogene 1997, 15:1059–1067
12. Johnson RL, Rothman AL, Xie J, Goodrich LV, Bare JW, Bonifas JM, Quinn AG, Myers RM, Cox DR, Epstein Jr EH, Scott MP: Human homolog of patched, a candidate gene for the basal cell nevus syndrome. Science 1996, 272:1668–1671
13. Hahn H, Wicking C, Zaphiropoulous PG, Gailani MR, Shanley S, Chidambaram A, Vorechovsky I, Holmberg E, Unden AB, Gillies S, Negus K, Smyth I, Pressman C, Leffell DJ, Gerrard B, Goldstein AM, Dean M, Toftgard R, Chenevix-Trench G, Wainwright B, Bale AE: Mutations of the human homolog of Drosophila patched in the nevoid basal cell carcinoma syndrome. Cell 1996, 85:841–851
14. Gailani MR, Stahle-Backdahl M, Leffell DJ, Glynn M, Zaphiropoulos PG, Pressman C, Unden AB, Dean M, Brash DE, Bale AE, Toftgard R: The role of the human homologue of Drosophila patched in sporadic basal cell carcinomas. Nat Genet 1996, 14:78–81
15. Unden AB, Holmberg E, Lundh-Rozell B, Stahle-Backdahl M, Zaphiropoulos PG, Toftgard R, Vorechovsky I: Mutations in the human homologue of Drosophila "patched" (PTCH) in basal cell carcinomas and the Gorlin syndrome: different in vivo mechanisms of PTCH inactivation. Cancer Res 1996, 56:4562–4565
16. Chidambaram A, Goldstein AM, Gailani MR, Gerrard B, Bale SJ, DiGiovanna JJ, Bale AE, Dean M: Mutations in the human homologue of the Drosophila patched gene in Caucasian and African-American nevoid basal cell carcinoma syndrome patients. Cancer Res 1996, 56:4599–4601
17. Wolter M, Reifenberger J, Sommer C, Ruzicka T, Reifenberger G: Mutations in the human homologue of the Drosophila segment polarity gene patched (PTCH) in sporadic basal cell carcinomas of the skin and primitive neuroectodermal tumors of the central nervous system. Cancer Res 1997, 57:2581–2585
18. Aszterbaum M, Rothman A, Johnson RL, Fisher M, Xie J, Bonifas JM, Zhang X, Scott MP, Epstein Jr EH: Identification of mutations in the human PATCHED gene in sporadic basal cell carcinomas and in patients with the basal cell nevus syndrome. J Invest Dermatol 1998, 110:885–888
19. Pawsey SA, Magnus IA, Ramsay CA, Benson PF, Giannelli F: Clinical, genetic and DNA repair studies on a consecutive series of patients with xeroderma pigmentosum. Q J Med 1979, 48:179–210
20. Robbins JH: Xeroderma pigmentosum. Defective DNA repair causes skin cancer and neurodegeneration [clinical conference]. JAMA 1988, 260:384–388
21. Bodak N, Queille S, Avril MF, Bouadjar B, Drougard C, Sarasin A, Daya-Grosjean L: High levels of patched gene mutations in basal-cell carcinomas from patients with xeroderma pigmentosum. Proc Natl Acad Sci USA 1999, 96:5117–5122
22. Wei Q, Matanoski GM, Farmer ER, Hedayati MA, Grossman L: DNA repair capacity for ultraviolet light-induced damage is reduced in peripheral lymphocytes from patients with basal cell carcinoma [see comments]. J Invest Dermatol 1995, 104:933–936
23. Dahl E, Aberg M, Rausing A, Rausing EL: Basal cell carcinoma. An epidemiologic study in a defined population. Cancer 1992, 70:104–108
24. Xie J, Johnson RL, Zhang X, Bare JW, Waldman FM, Cogen PH, Menon AG, Warren RS, Chen LC, Scott MP, Epstein Jr EH: Mutations of the PATCHED gene in several types of sporadic extracutaneous tumors. Cancer Res 1997, 57:2369–2372
25. Franceschi S, Levi F, Randimbison L, La Vecchia C: Site distribution of different types of skin cancer: new aetiological clues. Int J Cancer 1996, 67:24–28
26. Bastiaens MT, Hoefnagel JJ, Bruijn JA, Westendorp RG, Vermeer BJ, Bouwes Bavinck JN: Differences in age, site distribution, and sex between nodular and superficial basal cell carcinoma indicate different types of tumors. J Invest Dermatol 1998, 110:880–884
27. Ingham PW: Transducing Hedgehog: the story so far. EMBO J 1998, 17:3505–3511
28. Wicking C, Shanley S, Smyth I, Gillies S, Negus K, Graham S, Suthers G, Haites N, Edwards M, Wainwright B, Chenevix-Trench G: Most germ-line mutations in the nevoid basal cell carcinoma syndrome lead to a premature termination of the PATCHED protein, and no genotype-phenotype correlations are evident. Am J Hum Genet 1997, 60:21–26
29. Marigo V, Davey RA, Zuo Y, Cunningham JM, Tabin CJ: Biochemical evidence that patched is the Hedgehog receptor. Nature 1996, 384:176–179